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Genomic Structure, Gene Expression, and Promoter Analysis of Human Multidrug Resistance-Associated Protein 7

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Key Words
Multidrug resistance · ABC transporter · MRP7 · Genomic organization · Promoter analysis

Abstract
The multidrug resistance-associated protein (MRP) subfamily transporters associated with anticancer drug efflux are attributed to the multidrug-resistance of cancer cells. The genomic organization of human multidrug resistance-associated protein 7 (MRP7) was identified. The human *MRP7* gene, consisting of 22 exons and 21 introns, greatly differs from other members of the human MRP subfamily. A splicing variant of human *MRP7*, *MRP7A*, expressed in most human tissues, was also characterized. The 1.93-kb promoter region of *MRP7* was isolated and shown to support luciferase activity at a level 4- to 5-fold greater than that of the SV40 promoter. Basal *MRP7* gene expression was regulated by 2 regions in the 5′-flanking region at –1,780–1,287 bp, and at –611 to –208 bp. In Madin-Darby canine kidney (MDCK) cells, MRP7 promoter activity was increased by 226% by genotoxic 2-acetylaminofluorene and 347% by the histone deacetylase inhibitor, trichostatin A. The protein was expressed in the membrane fraction of transfected MDCK cells.

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Introduction

Clinical drug resistance to chemotherapeutic agents is a major obstacle in cancer therapy. Multidrug resistance (MDR) describes the phenomenon of simultaneous resistance to unrelated drugs used in chemotherapy. In humans, the P-glycoprotein (P-gp) and the multidrug resistance-associated protein (MRP) genes are the 2 adenosine triphosphate-binding cassette (ABC) transporter genes identified in MDR gene families to date. In 1992, from a doxorubicin-selected human small-cell lung cancer cell line, Cole et al. [4] identified a second type of drug efflux pump, multidrug-resistance protein 1 (MRP1). The drug resistance profile of cells transfected with MRP1 expression vectors was similar but not identical to that of cells that overexpress P-gp (MDR1) [5, 25]. In contrast to P-gp, MRP1 is an organic anion transporter that transports a wide range of organic anionic conjugates, such as glutathione, leukotriene C_4, and activated aflatoxin B1 [14, 22, 24, 28].

Seven members of the MRP subfamily have been identified so far. MRP2 was originally cloned in 1996 and is known as the canalicular multispecific organic anion transporter (cMOAT) [3, 12, 37]. Immunofluorescence studies have demonstrated that the MRP2 protein is absent from the liver of GY/TR and EHBR rats (rat

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strains lacking cMOAT) and from the liver of patients with Dubin-Johnson syndrome [15, 26, 29, 38]. In addition to MRP1 and MRP2, there are at least 5 other MRP homologs expressed in humans: MRP3, MRP4, MRP5, MRP6, and MRP7 [11, 19, 20]. Unlike MRP1 and MRP2, MRP3 prefers glucuronate to GSH conjugates as a substrate [9]. MRP4 and MRP5 act as cellular efflux pumps for nucleoside analogs [27, 30, 42]. MRP6 is highly expressed in the liver and kidney, but does not play a role in the resistance of the resistant cells analyzed [20]. MRP7 was only recently discovered, and its biological function has not yet been characterized. MRP7 encodes a 158-kD protein in reticulocyte lysates, and maps to chromosome 6p12–21 by in situ hybridization; its transcript is expressed at very low levels in a variety of human tissues [11]. The potential involvement of MRP6 and MRP7 in drug resistance remains unclear.

Most ABC transporters contain 2 types of structural domains: a hydrophobic, polytopic membrane-spanning domain (MSD), and a hydrophilic, cytosolic nucleotide-binding domain (NBD). The highly conserved NBDs of ABC family members share 2 sequence motifs, designated 'Walker A' and 'Walker B', with many other nucleotide-binding proteins [41]. These Walker sequences are separated by a stretch of about 120–170 amino acids (a.a.), including a short (12–13 a.a.) peptide motif, called the ABC transporter 'signature' region, which is in fact diagnostic for these proteins [32]. Based on the degree of amino acid identity and predicted topologies, the MRP subfamily proteins have been classified into 2 groups. Members of 1 group – MRP1, MRP2, MRP3, and MRP6 – have the extra N-terminal membrane-spanning domain (also called TMD_0) that is lacking in the other group – MRP4 and MRP5. Therefore, both MRP4 and MRP5 are smaller than MRP1. In addition, both MRP4 and MRP5 have less than 40% amino acid identity with MRP1 [1, 39].

To search for novel members of the MRP family, we used conserved sequences of the family as primers for PCR amplification and independently isolated a different MRP7 member, an alternatively spliced variant of MRP7. Because of its homology with MRP7, we named it MRP7A. To characterize these 2 different MRP7 members, we analyzed their tissue distributions and found that they are tissue specific. We also compared their cDNA sequences with the genomic sequence and determined the genomic structure. To understand the gene regulation of MRP7, we isolated a 1.93-kb promoter region and found that the promoter activity could be upregulated by genotoxic 2-acetylaminofluorene (2-AAF) and the histone deacetylase inhibitor trichostatin A (TSA). The recombinant protein of MRP7A was expressed in the membrane fraction of transfected Madin-Darby canine kidney (MDCK) cells.

Materials and Methods

Isolation of the Full-Length cDNA of Human MRP7A

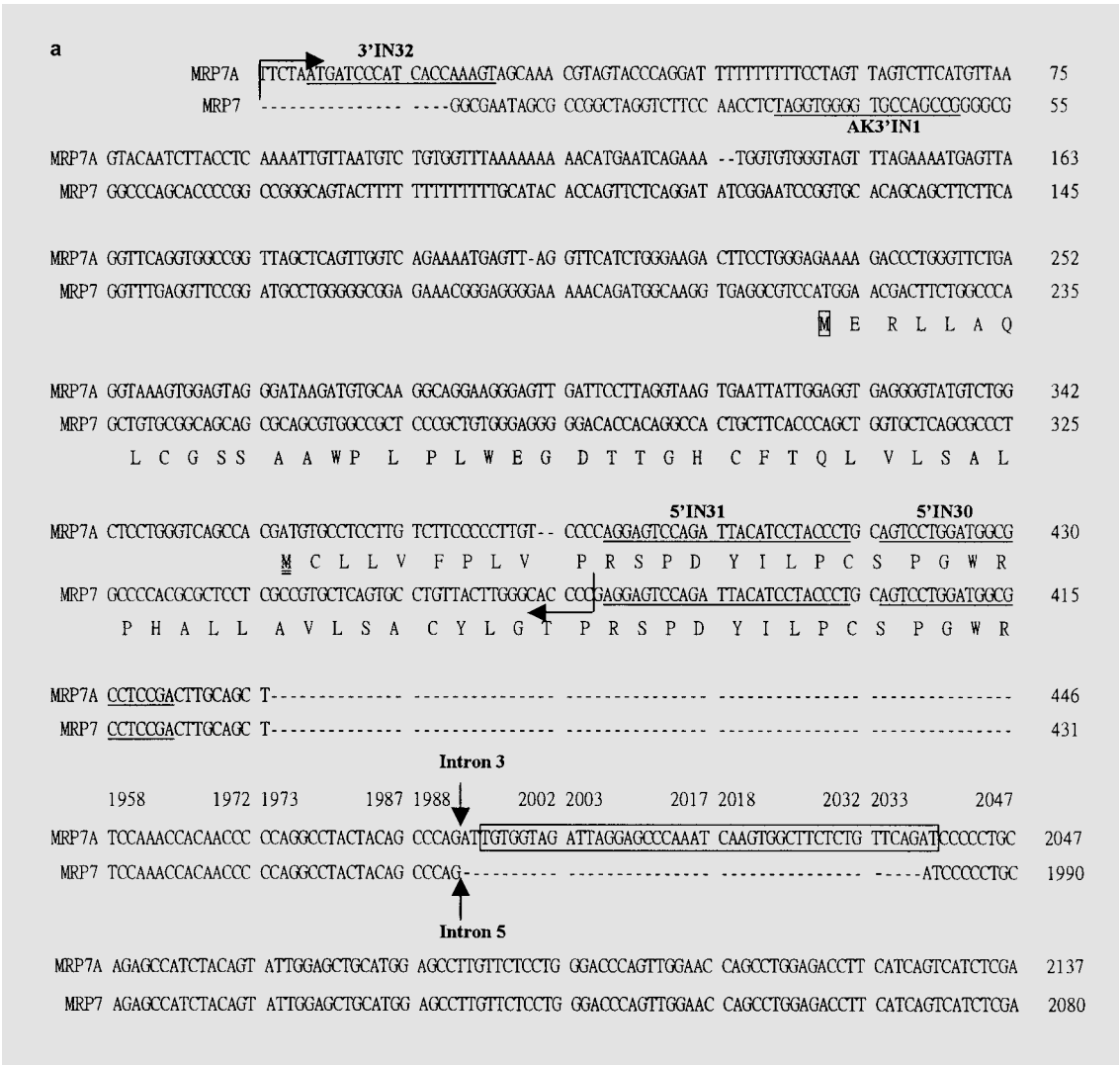
A cDNA fragment was amplified from human small intestine Marathon-Ready™ cDNA (Clontech Laboratories, Palo Alto, Calif., USA) by a pair of degenerate primers, Deg-S (5′-GAGAAGGTCGG-CATCGTGGG(AGTC)CG(AGTC)AC(AGTC)GG-3′) and Deg-Ans (5′-GTCCACGGCTGC(AGTC)GT(AGTC)GC(TC)TC(AG)-TC-3′), designed according to the conserved region in the C-terminal ABC region of human MRP family member genes. The PCR product was cloned into the PCRI I vector (Invitrogen, Carlsbad, Calif., USA) and then sequenced (sequencer model 377A, Applied Biosystems, Foster City, Calif., USA). 3′ RACE was carried out using human small intestine Marathon-Ready cDNA (Clontech Laboratories) with an oligo-d $(T)_{15}$ primer (Promega, Madison, Wisc., USA) and an internal gene-specific primer located in the previously identified C-terminal ABC unit of human MRP7A. An 881-bp 3′-end cDNA fragment, including a poly-A tail and a partially known sequence, was identified through TA cloning and autosequencing. Isolation of the 5′-end 3,896-bp cDNA fragment was carried out through a series of inverse polymerase chain reactions. A full-length cDNA clone was isolated and designated MRP7A.

Quantitative PCR

To determine the tissue-specific expression of *MRP7* and *MRP7A*, sense primers specific for each transcript and common antisense primers were used for PCR amplification. The PCR fragments containing the nucleotide sequences located from nt 6 to 414 upstream of *MRP7A* and from nt 33 to 399 upstream of *MRP7* were amplified by PCR (fig. 1a). The transcriptional start sites of both *MRP7A* and *MRP7* are designated as the first nucleotides (+1). Different human tissue cDNAs (Clontech Laboratories) were used as templates. The primers used were an antisense primer common to both *MRP7A* and *MRP7*, 5′IN30 (nt 417–437 for *MRP7A*; nt 402–422 for *MRP7*), and the specific primers located at the 5′-end of *MRP7A* (3′IN32, nt 6–24 for *MRP7A*) and *MRP7* (AK3′IN1, nt 33–50 for *MRP7*), respectively. Nested PCR was performed with a nested antisense primer (5′IN31, nt 390–414 for *MRP7A*; nt 375–399 for *MRP7*) and the same sense primers specific to *MRP7A* (3′IN32) and *MRP7* (AK3′IN1) using the first PCR products diluted 50 times as the templates. The lengths of the nested PCR products were 409 bp for *MRP7A* and 367 bp for *MRP7*. The PCR was amplified for 20 cycles. The primer sequences for quantitative PCR were as follows: 3′IN32: 5′-ATGATCCCATCACCAAAGT-3′; AK3′IN1: 5′-TAGGTGGGGTGCCAGCCG-3′; 5′IN30: 5′-TCGGAGGCGC-CATCCAGGACT-3′; and 5′IN31: 5′-AGGGTAGGATGTAATCT-GGACTCCT-3′.

Isolation of the Promoter Region and Analysis of Promoter Activity

Four different plasmid DNA fragments were constructed to analyze their promoter activities. A 1.93-kb DNA fragment (PA) upstream of the transcriptional start site of *MRP7* (nt –1,780–159) was


a
3'IN32
MRP7A TTCTAATGATCCCAT CACCAAAGTAGCAAA CGTAGTACCCAGGAT TTTTTTTTTCCTAGT TAGTCTTCATGTTAA 75
MRP7 ---------- ----GGCGAATAGCG CCGGCTAGGTCTTCC AACCTCTAGGTGGGG TGCCAGCCGGGGCG 55
AK3'IN1
MRP7A GTACAATCTTACCTC AAAATTGTTAATGTC TGTGGTTTAAAAAAA AACATGAATCAGAAA --TGGTGTGGGTAGT TTAGAAAATGAGTTA 163
MRP7 GGCCCAGCACCCCGG CCGGGCAGTACTTTT TTTTTTTTGCATAC ACCAGTTCTCAGGAT ATCGGAATCCGGTGC ACAGCAGCTTCTTCA 145
MRP7A GGTTCAGGTGGCCGG TTAGCTCAGTTGGTC AGAAAATGAGTT-AG GTTCATCTGGGAAGA CTTCCTGGGAGAAAA GACCCTGGGTTCTGA 252
MRP7 GGTTTGAGGTTCCGG ATGCCTGGGGGCGGA GAAACGGGAGGGGAA AAACAGATGGCAAGG TGAGGCGTCCATGGA ACGACTTCTGGCCCA 235
M E R L L A Q
MRP7A GGTAAAGTGGAGTAG GGATAAGATGTGCAA GGCAGGAAGGGAGTT GATTCCTTAGGTAAG TGAATTATTGGAGGT GAGGGGTATGTCTGG 342
MRP7 GCTGTGCGGCAGCAG CGCAGCGTGGCCGCT CCCGCTGTGGGAGGG GGACACCACAGGCCA CTGCTTCACCCAGCT GGTGCTCAGCGCCCT 325
L C G S S A A W P L P L W E G D T T G H C F T Q L V L S A L
5'IN31
5'IN30
MRP7A CTCCTGGGTCAGCCA CGATGTGCCTCCTTG TCTTCCCCCTTGT-- CCCCAGGAGTCCAGA TTACATCCTACCCTG CAGTCCTGGATGGCG 430
M C L L V F P L V P R S P D Y I L P C S P G W R
MRP7 GCCCCACGCGCTCCT CGCCGTGCTCAGTGC CTGTTACTTGGGCAC CCCGAGGAGTCCAGA TTACATCCTACCCTG CAGTCCTGGATGGCG 415
P H A L L A V L S A C Y L G T P R S P D Y I L P C S P G W R
MRP7A CCTCCGACTTGCAGC T---------- ---------- ---------- ---------- ---------- 446
MRP7 CCTCCGACTTGCAGC T---------- ---------- ---------- ---------- ---------- 431
Intron 3
1958 1972 1973 1987 1988 2002 2003 2017 2018 2032 2033 2047
MRP7A TCCAAACCACAACCC CCAGGCCTACTACAG CCCAGATTGTGGTAG ATTAGGAGCCCAAAT CAAGTGGCTTCTCTG TTCAGATCCCCCTGC 2047
MRP7 TCCAAACCACAACCC CCAGGCCTACTACAG CCCAG---------- ---------- ---------- -----ATCCCCCTGC 1990
Intron 5
MRP7A AGAGCCATCTACAGT ATTGGAGCTGCATGG AGCCTTGTTCTCCTG GGACCCAGTTGGAAC CAGCCTGGAGACCTT CATCAGTCATCTCGA 2137
MRP7 AGAGCCATCTACAGT ATTGGAGCTGCATGG AGCCTTGTTCTCCTG GGACCCAGTTGGAAC CAGCCTGGAGACCTT CATCAGTCATCTCGA 2080
b
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14
1000 bp
850 bp
650 bp
500 bp
400 bp
300 bp
200 bp
100 bp
409 bp
367 bp

isolated from the human genomic clone AC021391 and subcloned into pGL3-Enhancer DNA containing a luciferase gene as a reporter gene. A series of fragments deleted from the 5′-end (nt −1,780 to −208) upstream of the transcriptional start site (PB, PC, and PD) were PCR-amplified using the PA DNA fragment as a template. These DNA fragments were similarly cloned into a pGL3-Enhancer vector. The constructs were used along with promoterless pGL3-Enhancer DNA as a negative control in the transfection of MDCK cells (American Type Culture Collection, Manassas, Va., USA). The pGL3-Promoter vector with the SV40 promoter was used as the positive control. Cells at a density of 3×10^5/well in a 6-well plate were cotransfected with 4 μg of plasmid DNA and 200 ng of pCMV-β-galactosidase plasmid as an internal control, using 5 μl of lipofectAMINE 2000 reagent (Gibcol™, Invitrogen). Five hours after transfection, the medium was replaced with fresh growth medium. Cells were collected, and their luciferase activity was analyzed according to the manufacturer's (Promega) instructions after 24 h of transfection. To test the regulation of promoter activity by different reagents, 2-AAF (Sigma Chemical, St. Louis, Mo., USA) or TSA (Sigma) was added to the transfected cells 24 h after transfection. Luciferase activity was analyzed after cells had been incubated with the reagents for 19 h.

Western Blot Analysis of the Expression of Human MRP7A

The expression of human *MRP7A* was analyzed by Western blot analysis of the total membrane fraction and the cytosol fraction of transfected MDCK cells. The human *MRP7A* expression vector was constructed by cloning full-length *MRP7A* cDNA with a 3′ His tag[?] into the expression vector pcDNA3.1 (–) (Invitrogen).

Cells were grown routinely at 37°C in Dulbecco's modified Eagle's medium (high glucose) (Gibcol, Invitrogen) containing 10% fetal bovine serum. The MDCK cells were transfected, with or without human *MRP7A* cDNA, by lipofectamins (Gibcol, Invitrogen) and then selected with G418 (Sigma). Transfected cells were treated with hypotonic buffer (10 m*M* Tris-HCl, pH 7.5; 10 m*M* KCl; 1.5 m*M* $MgCl_2$) and supplemented with protease inhibitors (0.1 *M* PMSF; 1 m*M* leupeptin; 0.3 m*M* aprotinin). The suspension was homogenized in a chilled Tenbroeck homogenizer and centrifuged at 800 *g* to remove nuclei and any remaining intact cells. A membrane-enriched fraction was prepared by ultracentrifugation of the supernatant at 100,000 *g*. Pellets were resuspended in 10 m*M* Tris-HCl (pH 7.5) buffer containing 125 m*M* sucrose.

Proteins were resolved on a 7% separating gel by PAGE and then transferred to polyvinylidene difluoride membranes (Millipore, Bedford, Mass., USA). For the detection of MRP7A, blots were incubated with monoclonal IgG, Anti-His (C-term)-HRP antibody (Invitrogen). Antibody binding was visualized with enhanced chemiluminescence detection (Amersham International, Little Chalfont, UK) and by exposure on Kodak X-OMAT film.

Results and Discussion

Isolation of the Human MRP7A Full-Length cDNA Clone

A full-length cDNA clone of 5,082 bp, including 359 bp of the 5′-untranslated region, 4,389 bp of the *MRP7A* coding region, and 334 bp of the 3′-untranslated region, was isolated from human small intestine (GenBank accession No. AY032599). Computer BLAST analysis of the human *MRP7A* gene against the *NCBI* gene database showed a similar cDNA clone, *MRP7* (accession No. BAA92227 [11]). *MRP7* was isolated from human spleen containing a 4,479-bp open reading frame (ORF). Sequence analysis demonstrated that *MRP7A* is an alternatively spliced variant of human *MRP7* as shown in figure 1a. *MRP7A* and *MRP7* have 2 major differences. First, *MRP*7 has a 45-bp deletion that corresponds to the cDNA sequence of *MRP7A* from nucleotides 1,995 to 2,039. Second, sequences of the cDNA clone, *MRP7*, showed an additional 374-bp sequence at its 5′-end that resulted in different sequences of 124 a.a. and 2 extra exons than *MRP7A* (genomic structure described below in fig. 3).

To prove whether splice variants are tissue-specific, we performed PCR analysis on both small intestine and spleen cDNA (Clontech Laboratories) using the primers on both the 5′- and 3′-ends of the deleted 45-bp fragment. The results show that spleen cDNA expressed only the 45-bp-deleted variant, while small intestine cDNA expressed both variants with and without the additional 45-bp insert (data not shown). These data indicate that the alternatively spliced variants are tissue specific. The junction site of the splicing variant is located at intron 3 and exon 4 (genomic structure described below). Therefore, exon 6 of *MRP7* is shorter than exon 4 of *MRP7A* by 15 a.a. at its 5′ end (fig. 1a, fig. 3). These 15 amino acids encode the link-

Fig. 1. Expression of human *MRP7A* and *MRP7* in different human tissues. a Alignment of nucleotide sequences of the human *MRP7* and *MRP7A* cDNA. Amino acid sequences were aligned with the MAP program through the BCM (Baylor College of Medicine) search launcher. A 374-bp difference between *MRP7A* and *MRP7* cDNA is indicated by the arrows (↱, ↳). The translation initiation site of *MRP7A* is indicated by a framed M, while the translation initiation site of *MRP7* is indicated by a double-underlined M. Underlined sequences were the primers used in the PCR analysis of tissue-specific transcripts. The 45-bp deletion at exon 6 of *MRP7* is boxed. The position of the exon-intron junction at the alternative splicing site (intron 3 for *MRP7A* or intron 5 of *MRP7*) is indicated by vertical arrows. b PCR analysis of the expression of human *MRP7A* and *MRP7* in different human tissues. Lanes 1, 2: spleen; lanes 3, 4: small intestine; lanes 5, 6: heart; lanes 7, 8: kidney; lanes 9, 10: liver; lanes 11, 12: lung; and lanes 13, 14: nuclease-free water (negative control). Primers specific for *MRP7A* were used in odd-numbered lanes. Primers specific for *MRP7* were used in even-numbered lanes. PCR products were separated by 1.5% agarose electrophoresis with a 1-kb plus marker (Gibcol™, Invitrogen), lane M.

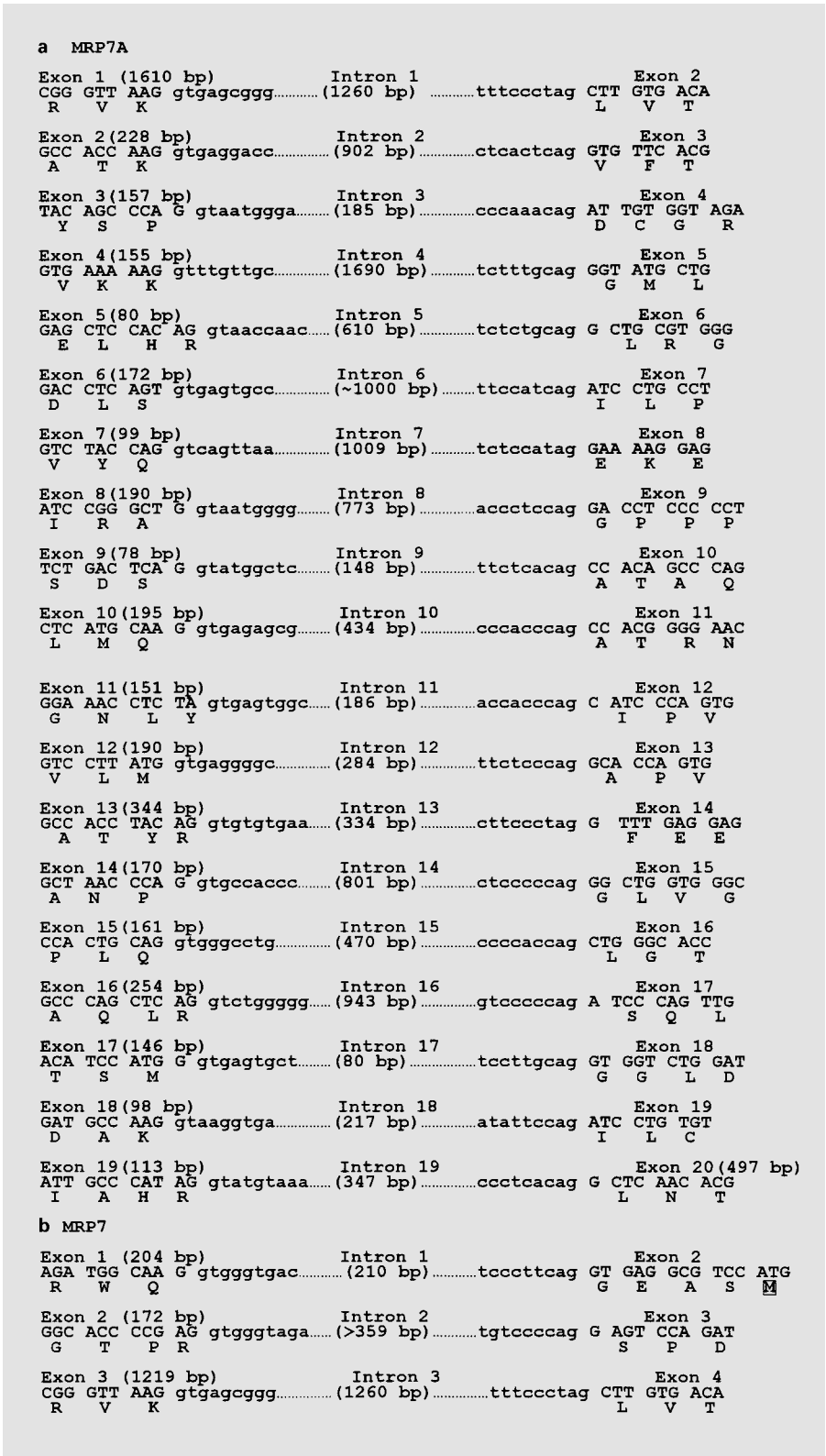


Fig. 2. Sequence of the intron/exon junctions of the human *MRP7A* gene (a) and human *MRP7* gene (b). Exon sequences are shown in uppercase letters, and intron sequences in lowercase letters. The encoded a.a. and its position are indicated below the exon sequences. The lengths of the introns are shown in parentheses. The translation initiation site of *MRP7* is indicated by M. The 3′-end of the 1,219-bp sequence of exon 3 of *MRP7* corresponds to the 3′-end of the 1,219-bp sequence of exon 1 of *MRP7A*. The intron-exon organization of *MRP7* corresponds to that of *MRP7A* after exon 3.

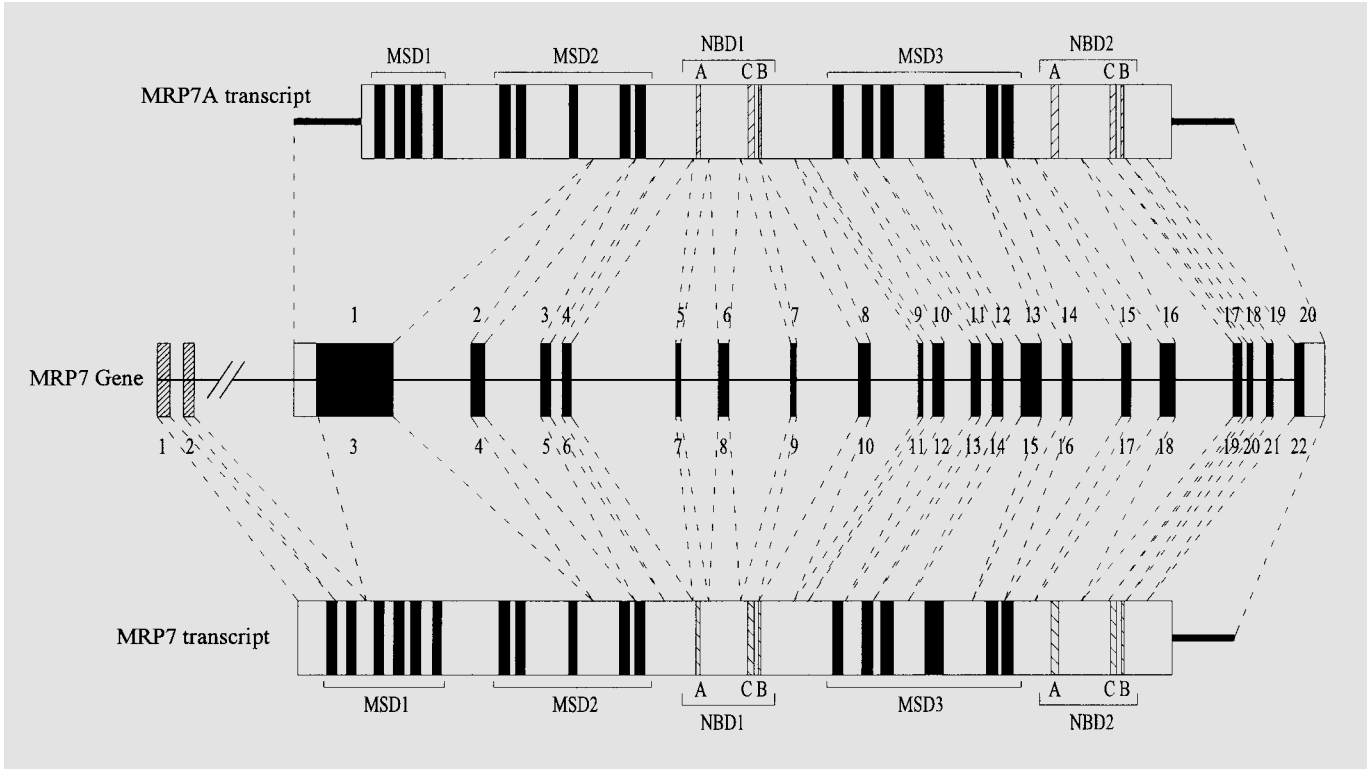


Fig. 3. Genomic organization of the *MRP7* gene and locations of exons for the 2 *MRP7* transcripts. Coding regions of exons are indicated by closed and diagonal boxes. Noncoding regions of exons are indicated by open boxes. The transmembrane helices included in each membrane-spanning domain (MSD1, MSD2, and MSD3) and nucleotide-binding domain (NBD1 and NBD2) are bracketed. Walker A and B motifs and the active transport family signature are denoted by A, B, and C, respectively.

age between MSD2 and NBD1, and are conserved in ABC transporters. Whether the 45-bp deletion makes any difference in their functions remains to be clarified.

BLAST analysis of the 374-bp sequence at the 5′ end of *MRP7* with the genomic sequence (accession No. AC021391) showed that *MRP7* contained 2 additional exons. In order to show that the 5′ alternatively spliced variants were tissue specific, we used the cDNAs of different tissues as templates to perform quantitative PCR reactions (fig. 1a, b). Primers used to specifically amplify either *MRP7A* or *MRP7* are shown in figure 1a. The antisense primers used were common to both transcripts, while 5′-sense primers were specific for each transcript. These specific primers specifically amplified 409-bp *(MRP7A)* and 367-bp *(MRP7)* PCR products. The result showed that only the spleen contained the *MRP7* transcript, while both the spleen and small intestine contained the *MRP7A* transcript. This result is consistent with what was observed in the analysis of the 45-bp deletion. In addition, *MRP7A* was expressed in most of the tissues analyzed, including the spleen, small intestine, heart, kidney, and liver, while *MRP7* was not detectable in any of the tissues except human spleen. The significance of the tissue-specific expression of these 2 splice variants for their biological function awaits further study. In the MRP subfamily, human *MRP3* and *MRP5* also have alternatively spliced variants. It will be interesting to determine whether these spliced variants play different roles in the physiological response to chemotherapy.

Genomic Structure of the Human MRP7 Gene

A human genomic clone (accession No. AC021391) containing the human *MRP7* gene was identified using a basic BLAST search. Two contigs in the BAC clone cover the entire human *MRP7* cDNA sequence. The human *MRP7A* gene spans more than 16 kb and consists of 20 exons and 19 introns (fig. 2). Exon 1 encodes the 359-bp 5′UTR of *MRP7A* mRNA and the first 1,251 bp of the

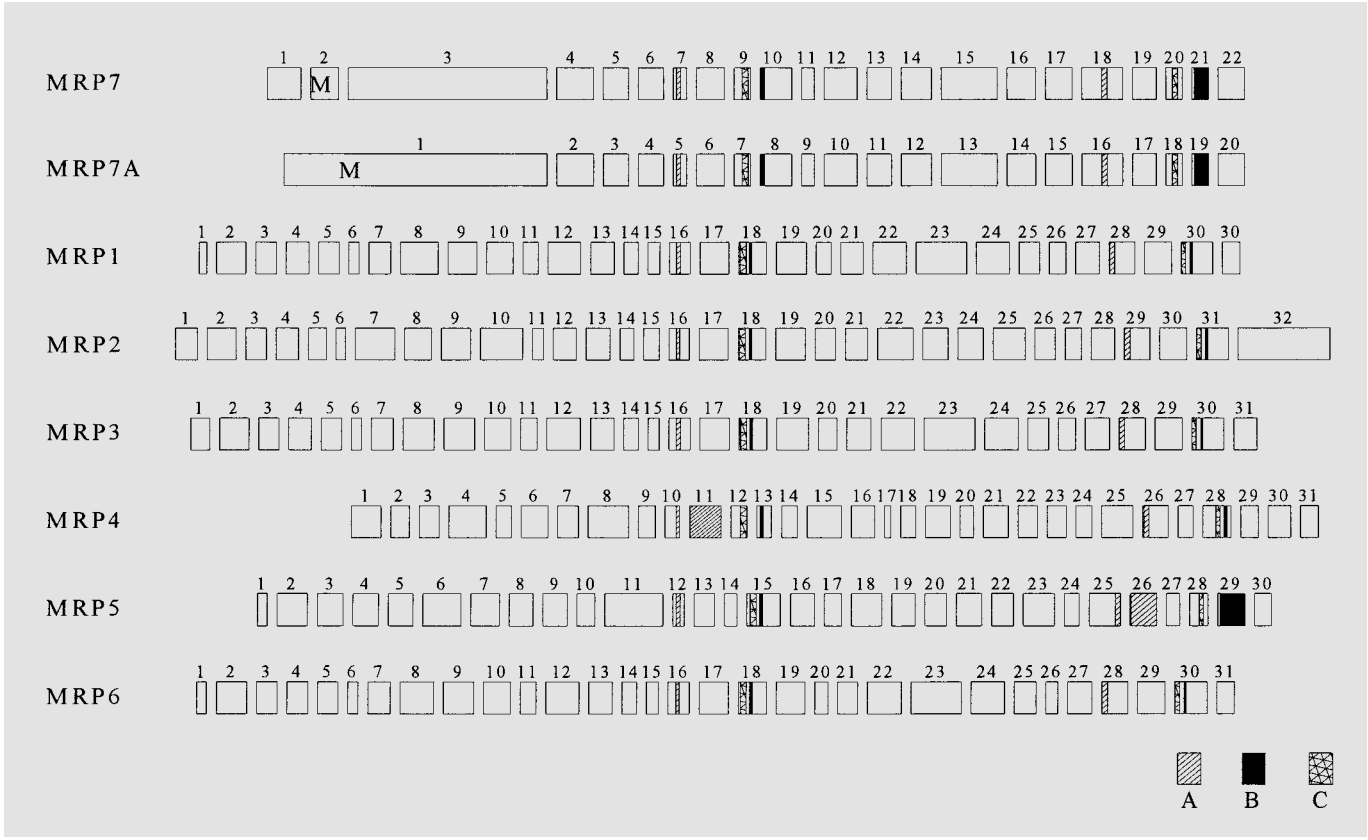


Fig. 4. Comparison of genomic structures of human MRP subfamily proteins. M is the location of the translation initiation site. The accession numbers used to analyze the genomic structures of the MRP proteins are as follows: *MRP1*, L05628 (cDNA clone), AC025277 (genomic clone), AC026452 (genomic clone); *MRP2*, U49248 (cDNA clone), AL133353 (genomic clone); *MRP3*, AF009670 (cDNA clone), AC004590 (genomic clone), AC005921 (genomic clone); *MRP4*, AF071202 (cDNA clone), AL356257 (genomic clone), AL157818 (genomic clone), AL139381 (genomic clone); *MRP5*, AF104942 (cDNA clone), AC068644 (genomic clone); *MRP6*, AF076622 (cDNA clone), AC020757 (genomic clone), U91318 (genomic clone), AC025778 (genomic clone), AC025277 (genomic clone); *MRP7*, BAA92227 (cDNA clone), AC021391 (genomic clone); and *MRP7A*, AY032599 (cDNA clone), AC021391 (genomic clone).

coding region. Exon 20 contains the last 163 bp of the coding sequence and the 334-bp 3′UTR. Intron sizes varied from 80 bp (intron 17) to 1,690 bp (intron 4). Of the 19 introns, 8 were class 0 (with the splice occurring between codons), 6 were class 1 (with the codon being interrupted between the first and second nucleotides), and 5 were class 2 (with the codon being interrupted between the second and third nucleotides). Sequences of the intron/exon junctions of the human *MRP7A* gene (fig. 2) conformed to the consensus sequence of the eukaryotic splice junctions [31].

The genomic organization of the *MRP7* gene and the locations of exons of the 2 *MRP7* transcripts are shown in figure 3. Each NBD of *MRP7A* mRNA was encoded by 4 exons, namely, exons 5–8 for NBD1 and exons 16–19 for NBD2, which is similar to NBD1 in *MRP4* and *MRP5*. In *MRP1* and *MRP2*, NBD1 is encoded by 3 exons [7, 38]. The extra N-terminal membrane-spanning domain (MSD1) in *MRP7* shows that *MRP7* belongs to the subgroup including *MRP1*, *MRP2*, *MRP3,* and *MRP6*. Compared with the structure of CFTR which lacks MSD1 and with the other MRP subfamily members with a MSD1 covered by 5–6 helices, *MRP7* might be a mediator from a common ABC transporter ancestor during the evolutionary process from CFTR to MRP or from MRP to CFTR [18].

The average size of the internal exons of human *MRP7A* is ~220 bp, which is larger than the average exon size of 137 bp for vertebrate genes [8]. Comparison of the human *MRP7* gene with other members of the human

MRP subfamily indicates that the genomic organization of the human *MRP7* gene greatly differs from those of the other MRP subfamily members (fig. 4). The length of exon 1 of the human *MRP7A* (exon 3 of *MRP7*) gene is quite large and covers 7 transmembrane helices, including the 4 helices in MSD1 and 3 out of the 5 helices in MSD2. The genomic structures of human *MRP4*, *MRP5*, and *MRP6* were also characterized and are presented here for comparison with the genomic structure of human *MRP7*. *MRP7* has 22 exons, far fewer than the other *MRP* genes, which contain 30–32 exons (fig. 4).

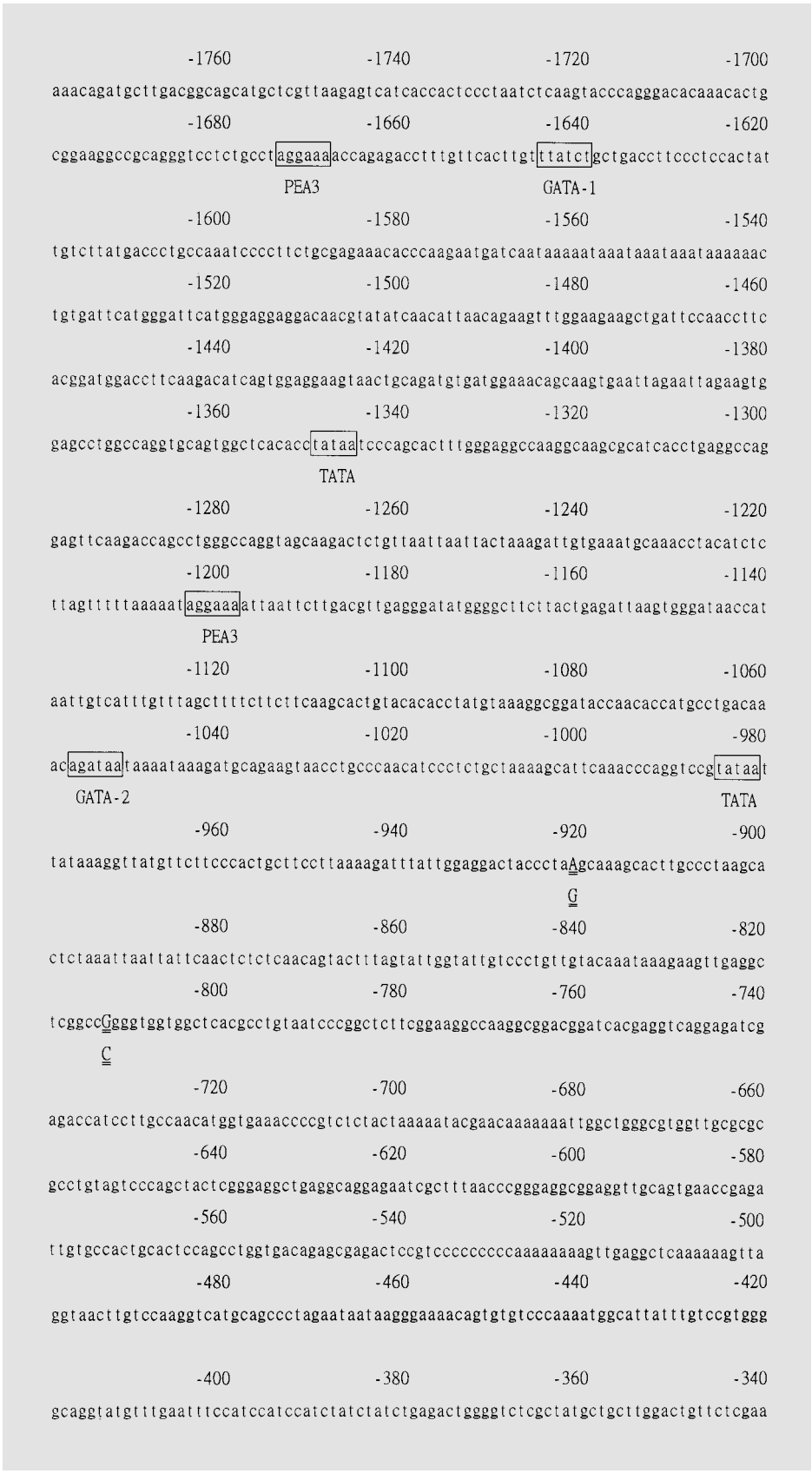
Analysis of Promoter Activity

We performed 5′ RACE and determined the transcriptional start site as shown in figure 5. The transcriptional start site was designated as the first nucleotide (+1). A 1.93-kb DNA fragment (nt –1,780–159) upstream of the translation initiation site of the human *MRP7* gene was isolated from the genomic clone. This fragment was subcloned into a pGL3-Enhancer vector containing the luciferase gene as a reporter gene. The 1.93-kb fragment supported 4- to 5-fold greater luciferase activity than that of the SV40 promoter (fig. 6b). There are several potential binding sites for transcription factors in this fragment, including 3 TATA boxes, 1 AP-2, 1 GATA-1, 1 GATA-2, and 6 GC-rich SP1 binding sites near the translation start site (fig. 5). The putative binding sites for transcription factors were identified with TRANSFAC of the HUSAR program based on the Wisconsin Genetic Computer Group (GCG) program. Thus, it is similar to other members of the MRP subfamily, i.e., *MRP1*, *MRP2*, and *MRP3*, which also contain GC-rich sites within the first 200 bases of their promoters [6, 21, 35, 36]. The multiple SP1 sites might be involved in human *MRP7* gene regulation, much like the SP1 and SP3 sites are involved in the regulation of the rat *MRP3* gene [40].

In order to further characterize this 1.93-kb fragment of the human *MRP7* gene and to determine sequences that are required for basal level gene expression, 3 different constructs with various deletions from the 5′-end of the fragment were transfected into MDCK cells (fig. 6a). As shown in figure 6b, luciferase activity was reduced to 50% when this fragment was deleted from nt –1,780 to –1,287. This activity was further reduced to 13% when the fragment was further deleted from nt –611 to –208. These results suggest the presence of positive regulatory elements responsible for the basal expression of the *MRP7* gene within the 2 regions. The TATA box, which is localized in –1,780/–1,287, might be important for the basal expression of the *MRP7* gene.

Based on the SNP database of NCBI, 2 single nucleotide polymorphism sites (SNPs) were also identified at nt –922 and –814 (shown in fig. 5). In previous studies, various mutations located in the coding regions were observed in MDR1, MRP1, and MRP2 transporter genes [13]. An SNP in exon 26 of the *MDR1* gene was correlated with P-gp levels and substrate uptake [10]. SNPs might provide a useful approach to individual therapy.

To investigate whether there are any reagents regulating promoter activity, the vector with the 1.93-kb of the *MRP7* 5′-flanking region ligated to the luciferase-containing plasmid pGL3-Enhancer was transfected into MDCK cells, and different reagents were added to the transfected cells. The promoter activities were analyzed after the cells had been treated with 2-AAF and TSA. The reporter gene activities of the *MRP7* promoter increased to 226% with 200 μ*M* of genotoxic 2-AAF, and 347% with 0.167 μ*M* of the histone deacetylase inhibitor TSA in MDCK cells (fig. 7). One study showed that human *MRP2* promoter activity in HepG2 cells treated with 200 μ*M* 2-AAF decreased to 60%, but that the activity of the human *MRP3* promoter increased to 165% [34]. Therefore, the effect of 2-AAF on the regulation of the *MRP*7 promoter seems to be similar to that on the human *MRP3* promoter (fig. 7) [16, 17]. The identity of the transcription factors involved in the induction by 2-AAF in the members of the MRP family is still unknown. PEA3 might be a possible candidate. The binding sites for PEA3 are present in the 2-AAF-responsive region of the rat *MRP2* gene [17], the 2-AFF-responsive region of the rat *MDR1b* gene [33], and the proximal promoter of the human *MRP2* gene [34]. There are 2 potential binding sites of PEA3 in the 1.93-kb *MRP7* 5′-flanking region. The responsive elements to 2-AFF in the promoter region of the human *MRP7* gene require further study. In addition, TSA diminished *MRP2* promoter activity but had no effect on *MRP3* promoter activity in HepG2 cells [34]. TSA is known to upregulate the transcription of the *c-myc* gene [25]. YY1 is a transcription factor associated with *c-myc*. Stöckel et al. [34] found that a YY1 binding site is located in the 5′-flanking region of the human *MRP2* gene, and that a 5′-deleted promoter construct of the human *MRP2* gene lacking the YY1 site was not inhibited by TSA. It is hypothesized that TSA enhances the transcription of *c-myc*, the increased *c-myc* associates with YY1, and then YY1 causes suppression of human *MRP2* transcription. Our results demonstrated that TSA upregulated *MRP7* promoter activity (fig. 7), which is distinct from its effect on the *MRP2* promoter. No YY1 binding sites were found in the 5′-flanking region of the human *MRP7* gene. It is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PEA3
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GATA-2 TATA
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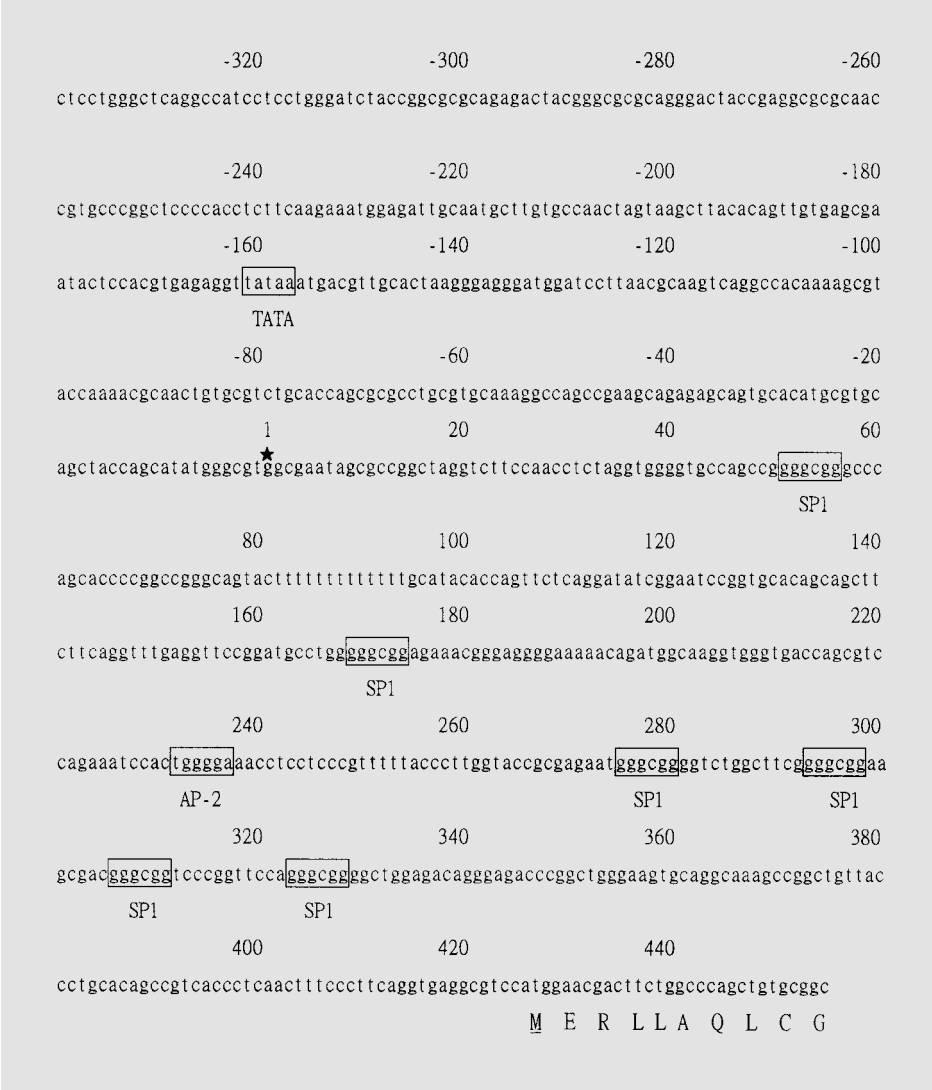

Fig. 5. Sequence of the 5′ noncoding region of the human *MRP7* gene. The 5′ upstream sequence along with the first 30 bases of the coding region is shown. The start codon of *MRP7* is underlined. The transcriptional start site is indicated by a star. Selected putative binding sites for transcription factors are boxed. The SNP sites located at nt −922 and −814 are capitalized and underlined.

possible that some regulatory elements and transcription factors other than YY1 and *c-myc* might be involved in the regulation of TSA on the *MRP7* promoter.

Western Blot Analysis of the Expression of Human MRP7A

The human *MRP7A* gene was expressed in transfected MDCK cells. The expressed protein was detected in the cell membrane fraction with a molecular weight of 158 kD by Western blot analysis (fig. 8). The MRP7A protein could not be detected in the cytosol fraction. This result indicates that MRP7A, like other MRP proteins, is a membrane protein [2].

In summary, we present *MRP7A*, an alternatively spliced variant of human *MRP7*, and show its specific expression in different tissues. We also characterize the genomic structure and promoter activity of the human *MRP7* gene. Defense against toxic compounds is a major

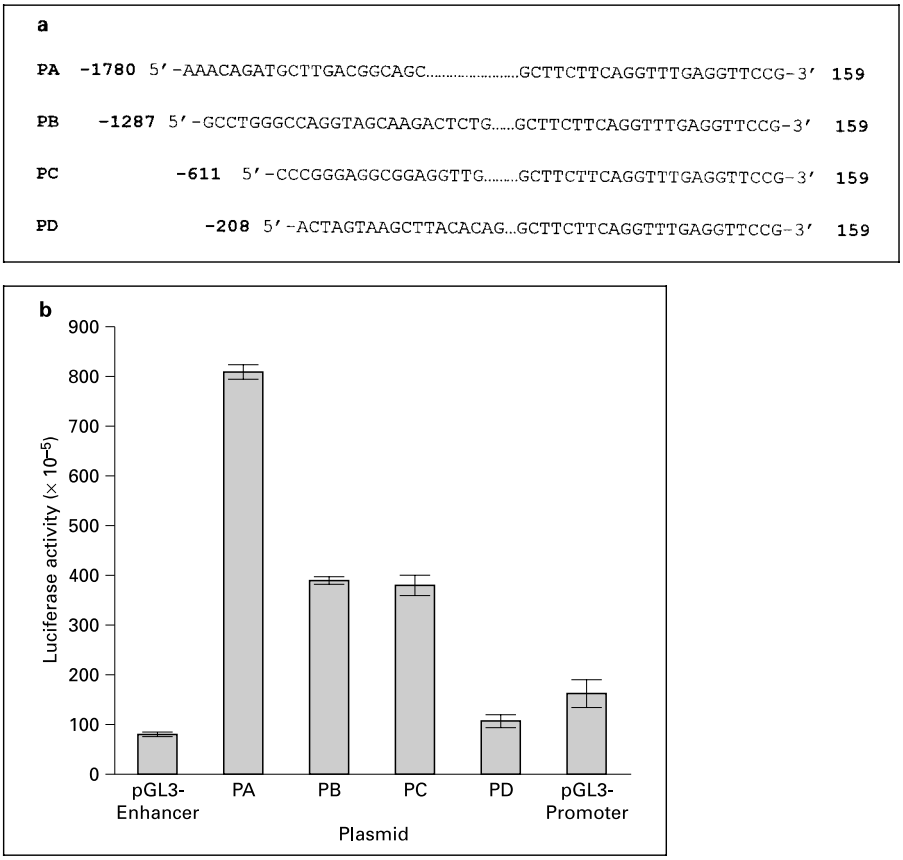

Fig. 6. Analysis of the luciferase activity of the human *MRP7* promoter. a Schematic display of 4 deletion constructs of the human *MRP7* promoter region. The 5′-flanking regions were cloned into the promoterless vector (pGL3-Enhancer). The numbers indicate the positions of the 5′ and 3′ ends of the constructs. The transcriptional start site was designated +1. b Luciferase activity of MDCK cells transfected with the above 4 deletion constructs. Data shown are the average of 3 independently transfected samples. The promoterless pGL3-Enhancer vector and pGL3-Promoter vector with SV40 were used as the negative and positive controls, respectively.

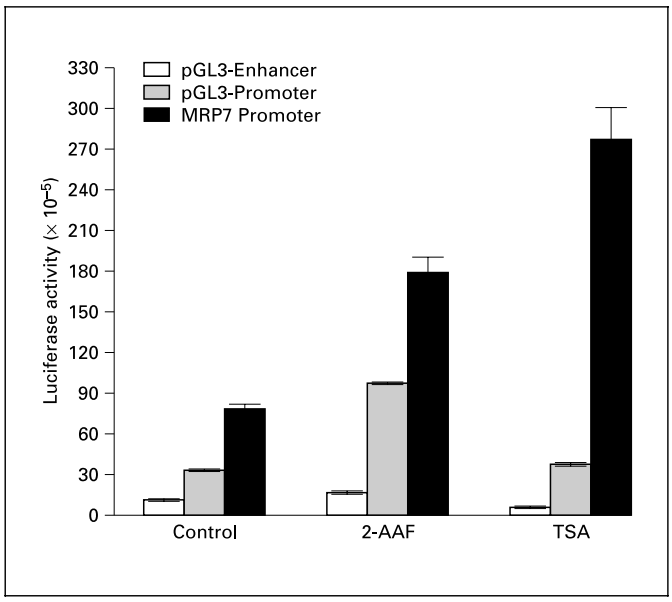

Fig. 7. Effect of 2-AAF and TSA on *MRP7* promoter activity. The promoterless pGL3-Enhancer vector and pGL3-Promoter vector with SV40 were used as the negative and positive controls, respectively.

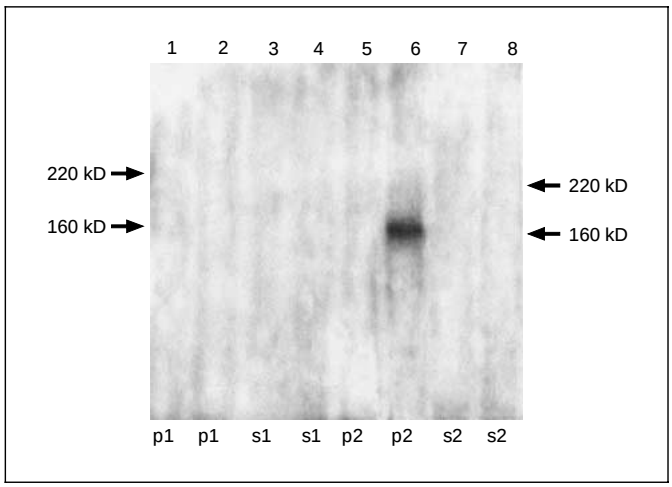

Fig. 8. Western blot analysis of human MRP7A protein in transfected MDCK cells with the anti-His (C-term)-HRP antibody. Lanes 1, 3, 5, and 7 were transfected with the pcDNA3.1 (–) vector; lanes 2, 4, 6, and 8 were transfected with pcDNA3.1 (–) with human *MRP7A* cDNA; p1 is the pellet after 800 *g* centrifugation; s1 is the supernatant after 800 *g* centrifugation; p2 is the pellet after 100,000 *g* centrifugation; and s2 is the pellet after 1% SDS extraction. Proteins were resolved on a 7% separating gel by PAGE.

concern of all living organisms, and ABC transporters play an essential role in this defense. Further biological function analysis of *MRP7A* and *MRP7* will provide information about whether these 2 molecules play some role in this defense. The information presented here can contribute to future studies.

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